LABELED ANTIMICROBIAL PEPTIDES FOR DETECTION OF MICROORGANISMS

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ABSTRACT

Antibodies have traditionally been used for pathogen detection but often lack stability and sensitivity. Current biosensor technologies using fluorescently labeled antibodies are generally capable of detection of 10^3 to 10^4 bacterial cells/ml. Antimicrobial peptides naturally bind to the lipopolysaccharide component of bacterial cell walls as part of their mode of action. Fluorescently labeled antimicrobial peptides (AMPs) were evaluated as a potential replacement of labeled antibodies in a sandwich assay. Due to their small size relative to antibodies peptides can bind to cell surfaces with greater density, yielding increased optical signal and improving sensitivity. The AMPs cecropin P1, SMAP29, and PGQ were synthesized with a C-terminal cysteine to which the fluorescent dye Cy5 was attached via maleimide linker chemistry. This method combines the specificity of a capture antibody with the increased sensitivity provided by using a labeled peptide as a detection molecule. Preliminary screening against *E. coli* O157:H7 using a whole-cell solution binding assay revealed that Cy5 cecropin P1 enhanced the detection 10-fold relative to a Cy5 labeled anti-*E. coli* O157:H7 antibody. Detection sensitivity of labeled antibody and peptide with a prototype immuno-magnetic bead biosensor revealed that Cy5 cecropin P1 again resulted in a 10-fold improvement in sensitivity. Correlation of peptide antimicrobial activity with detection of *E. coli* O157:H7 indicated that antimicrobial activity was not predictive of the sensitivity of the fluorescent assay. Labeled peptides also exhibit binding to spores and other bacteria, demonstrating potential utility for detection of other microorganisms.

1. INTRODUCTION

Antimicrobial peptides (AMPs) are part of the innate defense system found in all organisms to protect them from microbial infection and are classified primarily by secondary structure (Boman, 1995). They exhibit a relatively broad range of antimicrobial activity toward bacteria, fungi and viruses (Nicolas and Mor, 1995; Brogden, 2005). AMPs target gram-negative bacteria by binding non-specifically to the negatively charged lipopolysaccharide via electrostatic and van der waals interactions of both pathogenic and non-pathogenic organisms (Sawyer *et al.*, 1988; Piers *et al.*, 1994; Vorland *et al.*, 1999).

Much of the literature has focused on peptides as potential use as therapeutic agents or their interaction with artificial membranes and cells to elucidate mechanisms of antimicrobial activity. Recently AMPs have been immobilized on solid substrates for capturing and detecting microorganisms. Cecropin P1 (CP1) immobilization on maleic anhydride microplates using amine residues was reported for the capture of pathogenic and non-pathogenic strains of *E. coli* (Gregory and Mello, 2005). AMPs have also been immobilized via an engineered cysteine to control peptide orientation for the investigation of binding specificity of various microorganisms (Soares *et al.*, in press). Kulagina et al. immobilized AMPs onto glass slides using biotin-avidin chemistry for detection of *E. coli* O157:H7 and *Salmonella typhimurium* (Kulagina *et al.*, 2005) (Kulagina *et al.*, 2006). In these studies fluorescently labeled cells were detected directly or in a sandwich assay using a fluorescently labeled antibody. Detection limits *E. coli* and *Salmonella* were $5x10^4$ to $5x10^5$ and $1x10^5$ to $5x10^6$ CFU/ml, respectively. Enhanced detection of *E. coli* O157:H7 has been demonstrated with fluorescently labeled AMPs as a secondary label (Arcidiacono *et al.*, 2008).

There is a need for rapid, sensitive detection of pathogenic organisms, including environmental samples for presence of bacterial pollution, detection of bioagents, and food safety for the presence of spoilage and pathogenic organisms. Immuno-magnetic separation (IMS) is a common method used to capture cells with antibody conjugated paramagnetic beads and subsequently detected with a second labeled antibody. IMS has been used in food and environmental samples to capture *E. coli* O157:H7 (Shelton and Karns, 2001; Liu *et al.*, 2003; Parham *et al.*, 2003), *S. typhimurium* (Yu and Bruno, 1996) and *Bacillus stereothermophilus* (Blake and Weimer, 1997). Although detection of single organisms has been reported with IMS, a pre-enrichment step of up to 18 hours was required (Padhye and Doyle, 1991; Tsai *et al.*, 2000; Geng *et al.*, 2006). To achieve rapid detection, methods require an enhancement of sensitivity without the need for enrichment.

Antibodies are used in IMS because of their relative specificity and ability to minimize false positive results. Liu et al. demonstrated the ability to avoid cross-reactivity in a biosensor format distinguishing *E. coli* O157:H7 from *S. typhimurium, Listeria monocytogenes* and *Campylobacter jejuni* (Liu *et al.*, 2003). However, the elimination or reduction of cross-reactivity is not

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always possible and is dependent on the target organism and use of monoclonal or polyclonal antibodies. While possessing specificity, antibodies lack the sensitivity for direct measurement of low cell concentrations. The detection limit by fluorescence is generally 10^3 to 10^4 bacterial cells/ml in food matrices (DeMarco and Lim, 2001; Demarco and Lim, 2002; Geng *et al.*, 2006).

To address antibody limitations, AMPs were investigated as an alternative detection molecule. The class of linear, cationic peptides that form amphipathic α-helical structures upon cell binding are the focus of this study. CP1, SMAP29 and PGQ were used as labeled detection molecules. CP1 is found in nematodes from the stomachs of pigs (Pillai *et al.*, 2005) with antimicrobial activity against predominantly gram-negative bacteria. SMAP29 is from the cathelicidin family of peptides found in sheep (Bagella *et al.*, 1995). It is highly active against gram-positive and gram-negative bacteria as well as fungi. PGQ was isolated from frog skin but is the least active of the three investigated (Moore *et al.*, 1991) but is not well characterized.

Variants of these peptides were synthesized containing a C-terminal cysteine for selective attachment of the fluorescent dye Cy5. Labeled peptides were evaluated as a substitute for secondary antibodies in a whole-cell solution binding assay and a prototype magnetic bead immuno-capture sensing system. We hypothesized that enhanced sensitivity would be achieved using peptides for detection, taking advantage of the selectivity of binding by the capture antibodies and the high density binding of peptides to the cell surface. In the whole-cell binding assay, Cy5 labeled cecropin P1 (Cy5 CP1) was shown to enhance detection of *E. coli* O157:H7 10-fold relative to a Cy5 labeled anti-*E. coli* O157:H7 antibody. Use of Cy5 CP1 in an immuno-magnetic bead based biosensor also resulted in detection of 10^3 CFU/ml in buffer, a 10-fold improvement in sensitivity relative to a secondary antibody. While this data demonstrates utility with *E. coli* O157:H7, this technology may have application with additional microorganisms.

2. MATERIALS AND METHODS

2.1 Bacteria, Growth Conditions and Reagents.

Phosphate-buffered saline (PBS) consisted of 137 mM NaCl, 2.7 mM KCl, 4.4 mM Na_2HPO_4, and 1.4 mM KH_2PO_4. PBST was PBS supplemented with 0.05% Tween 20. *E. coli* O157:H7 (ATCC 43888), *E. coli* (ATCC 43827), and *Staphylococcus aureus* (ATCC 27217) was grown to mid-log in Luria broth (LB) at 37°C to OD_{600} = 1 (approximately 10^8 CFU/ml) and washed 2x in an equal volume of PBST before being resuspended in PBST. Cells were serially diluted 10-fold in PBST for detection sensitivity experiments. Bacterial spores (*Bacillus subtilis* (QM 1611), *B. stearothermophilus* (ATCC 12980), *B. megaterium* (QMB 1551), *B. atrophaeus* (NRRL B-4418) were prepared in water and washed 3x in PBST.

2.2 Detection Molecule Labeling

Antimicrobial peptides cecropin P1 (CP1) (Pillai *et al.*, 2005), SMAP29 (Bagella *et al.*, 1995), PGQ (Moore *et al.*, 1991), cecropin A (CA) (Sun *et al.*, 1998), ceratotoxin A (CTA) (Marchini *et al.*, 1993), CFP3 (Maloy and Kari, 1995), and a serine mutant of CP1 (Ser5 CP1) containing a C-terminal cysteine (Table 1) were chemically synthesized by SynPep Corp. (Dublin, CA). Peptides solubilized in phosphate buffered saline (PBS), pH 7.4 at 1 mg/ml were quantitated by BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL). A 3 molar excess of Tris(2-carboxyethyl)phosphine (Sigma Chemical Co., St. Louis, MO) was added to reduce the peptide. Peptide was fluorescently labeled at 90 nmol/vial Cy5 dye from Cy5 mono-reactive maleimide kit (Amersham Biosciences, Piscataway, NJ). Cy5 labeled peptides CP1, SMAP, and PQG were purified by RP_HPLC using a C_4 column, 250 x 4.6 mm, 5 µm pore size (YMC, Inc., Wilmington, NC). HPLC fractions were lyophilized, resuspended in PBST and analyzed by SDS-PAGE. Fractions with the labeled peptide were pooled and quantitated by reverse phase HPLC using unlabeled peptide as a standard curve.

Peptide	Amino acid sequence C-terminal variants	MW	pI	Charge	Target bacteria
CP1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPRC	3442	10.19	+5	gram negative
SMAP29	RGLRRLGRKIAHGVKKYGPTVLRIIRIAGC	3359	12.01	+9	gram positive gram negative
PGQ	GVLSNVIGYLKKLGTGALNAVLKQC	2560	9.63	+3	gram negative
CA	GGLKKLGKKLEGVGKRVFKASEKALPVAVGIKALGC	3651	10.2	+7	gram positive gram negative
CTA	SIGSALKKALPVAKKIGKIALPIAKAALPC	2972	10.3	+6	gram positive gram negative
CFP3	GFASFLGKALKAALKIGANMLGGTPQQC	2793	9.79	+3	gram negative
Ser5 CP1	SWLSsKTAKKLENSAKKRISEGIAIAIQGGPRC	3529	10.19	+5	unknown

Table 1. Characteristics of AMPs used as detection molecules. Cysteine peptide variants were synthesized with a C-terminal cysteine for attachment of the fluorescent Cy5 dye.

Affinity purified polyclonal antibody to *E. coli* O157:H7 was obtained from KPL Inc. (Gaithersburg, MD). One mg of antibody was reacted with a Cy5 mono-reactive maleimide kit (Amersham Biosciences) and purified according to the manufacturer's instructions.

2.3 Whole-cell Solution Binding Assay

A whole cell binding assay was used to screen peptides for detection sensitivity before moving to the immuno-capture assay (Figure 1). Cells were grown and prepared in PBST as described above. A 100 µl aliquot of serially diluted cells was added to 900 µl of PBST containing Cy5 peptide (5 µg/ml) or Cy5 anti-O157 antibody (1:1000 final dilution) in PBST and mixed on a Dynal rotary mixer (Dynal Biotech, Browndeer WI) at approximately 20 rpm (setting 20-25) for 30 minutes at ambient temperature. Cells were harvested at 10,000x*g* for 3 minutes, the supernatant removed with a pipet, and the pellet washed 3x with 1 ml PBST and spun as above. Cells were resuspended in 200 µl PBST and transferred to a black microplate (Nalge Nunc International, Rochester, NY). A 900 µl aliquot of peptide solution was added to 100 µl buffer without cells and assayed as the zero cell negative control. The microplate was imaged using the Storm 860 (Amersham Biosciences, Piscataway, NJ) using red fluorescence at 1000V PMT, 200 micron. The image was quantitated by TotalLab version 2003.03 software (Nonlinear Dynamics, Newcastle upon Tyne, UK).

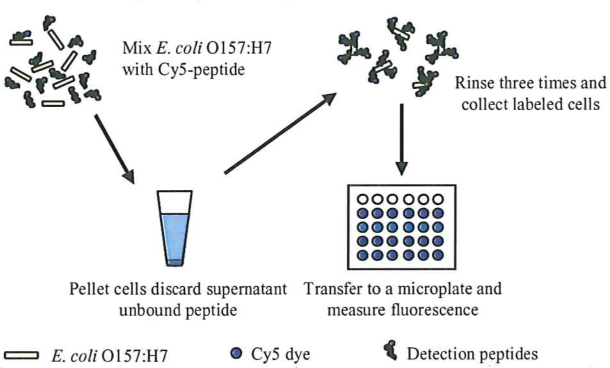

Figure 1. Schematic of whole cell solution binding assay.

2.4 Immuno-capture Biosensor Assays

Detection sensitivity of labeled antibody and peptides was determined using an immuno-capture method (Figure 2). Detection of *E. coli* O157:H7 were tested in buffer, milk and apple juice. Cells were grown and prepared in PBST as described above. 20 µl anti-*E. coli* O157 paramagnetic Dyna-beads (Dynal Biotech) were added to 1 ml of 10^3 to 10^6 CFU/ml cells in each solution and rotary mixed for 30 minutes. A zero cell sample of beads and sample was run as the negative control. Beads were collected and washed 3x with 1 ml

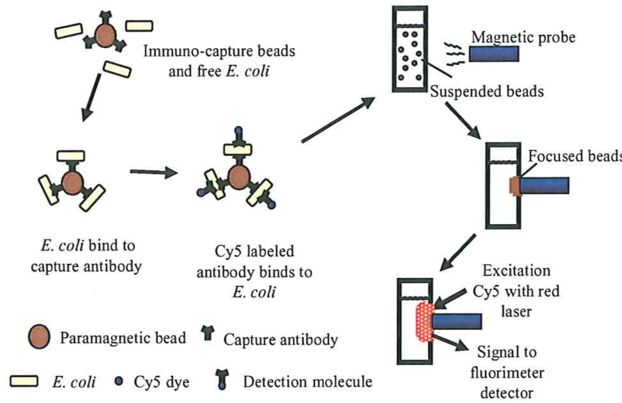

Figure 2. Schematic of magnetic immuno-capture assay. Peptide labeled cells on the beads are focused with a magnet to be read by laser at 650nm.

PBST. Cy5 peptides (5 µg) or Cy5 anti-O157 antibody (1:1000 dilution) in 1 ml PBST were added to cells captured on the magnetic beads. After 30 minutes of rotary mixing, beads were collected and washed in PBST. Beads were resuspended in 500 µl PBST for analysis on a second generation prototype magnetic focusing fiber optic fluorometer (Pierson Scientific Associates, Andover, MA). The system contains an excitation and emission fiber for the transmission of light at a wavelength of 650 nm. The excitation fiber delivered laser excitation light to the chamber and the emission fiber carried the emitted fluorescence light for measurement. The fibers were interfaced with a sample chamber that held a standard disposable semi micro-cuvette. The custom built chamber consisted of a metal probe that housed the fibers and a magnetic holder that securely positioned the probe at the cuvette wall. The holder also generated the magnetic field required to pull the paramagnetic microspheres out of solution to a spot in front of the excitation and emission fibers where the fluorescence was measured.

3. RESULTS AND DISCUSSION

3.1 Peptide Labeling

Peptides synthesized with a C-terminal cysteine were labeled with the fluorescent dye Cy5 via maleimide linker chemistry. The Cy5 mono-reactive maleimide kit was designed for one mg of antibody and modifications were necessary for peptide labeling. One mg of peptide failed to label with high efficiency. Peptide quantity was reduced to 90 nmol per reaction, comparable to the moles of labeling sites found on the antibody. HPLC purification was necessary due to the similar size of the peptides relative to that of free dye. The degree of peptide labeling with Cy5 dye was peptide dependent, but was generally about 50%. Labeled SMAP did not

separate well from free Cy5 dye on HPLC. However, any free dye present would not have contributed to the fluorescent signal in these binding studies, since unconjugated dye alone does not bind to cells or magnetic beads.

3.2 Detection

There are many methods for detection of food pathogens that employ labeled antibodies as detection molecules. Antibodies possess target specificity, but also have the limitations of insufficient detection sensitivity and instability. Using the same antibody for both capture and detection may result in competition for the same epitopes, causing steric hindrance and reduced sensitivity. Due to these factors, antimicrobial peptides were evaluated as alternative detection molecules in an immuno-capture assay to improve sensitivity. Peptides alone are not suitable for detection of microorganisms, due to their mode of binding non-selectively to the negatively charged LPS. Specificity for a particular organism would be addressed during target capture, using the selectivity of the capture antibody.

3.3.1 Peptide Screening by Solution Binding Assay

To determine the detection sensitivity of *E. coli* O157:H7, 5 μg/ml labeled peptides were tested in solution against 10^6 to 10^4 CFU/ml cell concentrations and compared to a 1:1000 dilution of Cy5-anti O157 antibody. Improved detection of *E. coli* O157:H7 using a labeled AMP in place of an antibody was demonstrated (Table 2). Of the three peptides evaluated, Cy5 CP1 was the most sensitive. With a detection sensitivity of 10^4 CFU/ml, Cy5 CP1 exhibited a 10-fold improvement over the Cy5 antibody detection of 10^5 CFU/ml. Neither Cy5 SMAP nor Cy5 PGQ improved sensitivity, detecting 10^5 and 10^6 CFU/ml respectively. While it was not determined if Cy5 CP1 could detect fewer cells, it appears that 10^4 CFU/ml may be approaching the sensitivity limit. Note that while Cy5 SMAP signal at 10^6 CFU/ml is 40x greater than Cy5 CP1, it as not as sensitive. Free, unconjugated Cy5 dye did not exhibit cell binding (data not shown).

3.3.2 Immuno-detection

Detection sensitivity of *E. coli* O157:H7 by Cy5 CP1 peptide and anti-O157 antibody was also determined using a prototype immuno-capture biosensor. Various concentrations of cells (10^6 to 10^3 CFU/ml) were captured on anti-O157 antibody conjugated magnetic beads and detected with Cy5 labeled antibody (1:1000 dilution) or Cy5 CP1 (5 μg/ml) (Table 3). As seen in the solution binding assay, Cy5 CP1 had a minimum of 10-fold more sensitivity than the Cy5 antibody (10^4 and 10^5 CFU/ml respectively). Cy5 CP1 also had a net positive signal at 10^3 CFU/ml cells, suggesting further enhancement in detection sensitivity. Cy5 SMAP and PGQ detection in the immuno-capture assay did not improve sensitivity relative to the antibody. Detection in milk using Cy5 CP1 was not as sensitive as the antibody. Detection in apple juice was improved a minimum of 10-fold relative to antibody and was similar to the results in buffer.

		Normalized net fluorescence			
Labeled detection molecule	**Net fluorescence 10^6 CFU/ml**	**10^6**	**10^5**	**10^4**	**Detection limit (CFU/ml)**
Cy5 antibody	6458	1.00	0.14	0	10^5
Cy5 CP1	3022	1.00	0.27	0.12	10^4
Cy5 SMAP	122283	1.00	0.11	0.01	10^5
Cy5 PGQ	1163	1.00	0.13	nc*	Inconclusive; low signal:noise

Table 2. Comparison of labeled molecules for detection of the food pathogen *E. coli* O157:H7 in solution binding assay. The labeled peptide Cy5 CP1 shows improved detection compared to the Cy5 anti-O157 antibody.
*nc – not conclusive
Net signal 10^6 CFU/ml normalized to 1.00 for each peptide. Net 10^5, 10^4 fluorescence adjusted based on 10^6.

There was a large amount of variability for Cy5 CP1 making it difficult to definitively determine a detection limit. The cause of inconsistency is not known, but did not appear to be instrument related since the antibody data was relatively consistent. Unlike labeled antibody, Cy5 CP1 did not respond proportionally to cell concentration. There was little statistical difference in signal between cell concentrations throughout the range tested. The zero cells control also exhibited variability, possibly caused by incomplete removal of unbound label

	Magnetic immuno-capture biosensor detection limit (CFU/ml)		
Detection molecule	Buffer	Apple juice	Milk
Cy5 antibody	10^4	10^4	10^4
Cy5 CP1	$\leq 10^3$	$\leq 10^3$	10^5
Cy5 SMAP	10^5	nd	nd
Cy5 PGQ	10^6	nd	nd

Table 3. Comparison of labeled molecules for detection of food pathogen *E. coli* O157:H7 on prototype biosensor. The labeled peptide Cy5 CP1 shows improved detection compared to the Cy5 anti-O157 antibody. (nd – not determined)

when washing the magnetic beads. Changes in peptide solution may also affect interaction with the beads. Further efforts are needed to improve assay to assay consistency.

In contrast to Cy5 antibody, there was significant non-specific binding of Cy5 CP1 to the magnetic beads. For 10^6 CFU/ml cells non-specific binding resulted in a signal to noise ratio of about 2. This ratio fell with decreasing cell concentration, significantly reducing net signal and possibly overall sensitivity. Inclusion of non-ionic detergent did not effectively reduce the signal. Free Cy5 dye did not exhibit binding to the magnetic beads; background is therefore thought to be due to non-specific peptide binding (data not shown). Background reduction would improve signal to noise and possibly sensitivity. A number of blocking agents were investigated to reduce non-specific binding, including bovine serum albumin (BSA), non-fat dry milk (NFDM), fetal bovine serum (FBS), and casein. To screen blocking ability the compounds were tested against beads without cells. 2.5% casein, 2% BSA and 0.2% NFDM reduced non-specific binding of labeled peptide to beads up to 64, 40-50, and 55% respectively. 10% FBS was not a suitable blocker (16% reduction). To determine blocking ability in the detection assay, the blocking agent was added to beads after cell capture. However, when testing casein as a blocker in the presence of 10^6 CFU/ml cells, Cy5 CP1 was prevented from cell binding. Sensitivity while blocking with BSA and NFDM has not yet been determined.

While the research reported here focused on detection of a food pathogen *E. coli* O157:H7, the same technology may be applicable for additional microorganisms. Several other Cy5 labeled peptides including CTA, CPF3 and CA have been shown to bind to gram negative (non-pathogenic *E. coli*) and gram positive (*Staphylococcus aureus*) bacteria. In addition, Cy5 labeled CP1 and SMAP exhibited binding to various *Bacillus* spp spores (Figure 3).

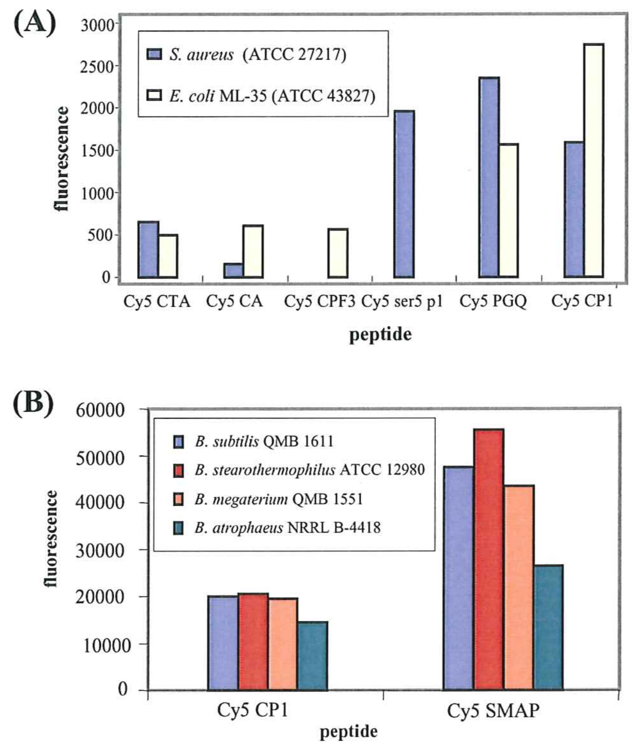

Figure 3. Labeled peptides tested in solution binding assays against other microorganisms. (A) 5 ug/ml unpurified Cy5 peptides binding against gram positive *S. aureus* and gram negative non-pathogenic *E. coli,* each at 10^6 CFU/ml. Peptides bind to gram positive and gram negative organisms with different affinities. Free Cy5 dye does not bind non-specifically to the cells. (B) Binding of 5 ug/ml purified Cy5 CP1 and Cy5 SMAP to various *Bacillus* spp spores at 10^7 CFU/ml. Affinity of Cy5 CP1 to the different spores is similar, while Cy5 SMAP binding is more variable.

3.4 Optimization

Although Cy5 CP1 improved sensitivity when compared to a labeled antibody, the assay method has not been optimized. Two parameters for optimization are possible; increasing sensitivity and reducing assay time. First, improved sensitivity may be achieved by greater labeling efficiency or increasing the quantity of Cy5 peptide in the assay. At 50% efficiency, Cy5 CP1 labeling could be improved to deliver more label to the cell surface. In addition, using an increased concentration of peptide solution might boost the amount of label binding to the cells. The solution binding assay with *E. coli* O157:H7 revealed a linear dose response curve using 0.5 to 10 μg/ml with purified Cy5 CP1. This was an effort to determine the concentration of labeled peptide required to saturate the cells to generate maximum signal; however, saturation was not achieved. At cell densities of 10^5 and 10^7 CFU/ml, greater fluorescent signal was observed as the quantity of peptide increased up to 10 μg/ml (data not shown). Increasing peptide concentration may further enhance sensitivity, since 5 μg/ml Cy5 CP1 did not achieve cell saturation. However, because the peptide quantity to saturate $<10^5$ CFU/ml has not been determined, it is not known whether increased peptide concentration will improve sensitivity.

Bactericidal activity appears to be an important factor influencing detection sensitivity. Activity was tested under conditions simulating the whole-cell solution-binding assay to determine if cell lysis was occurring during the 30 minute peptide-cell incubation. Although Cy5 SMAP had a much greater signal at a cell concentration of 10^6 CFU/ml, it was not as sensitive as Cy5 CP1. Following a dramatic reduction at 10^5

CFU/ml, Cy5 SMAP signal was completely lost at 10^4 CFU/ml. This may be attributed to activity, since Cy5 SMAP caused a 5-log reduction in cell concentration within 10 minutes (data not shown). The 30 minute peptide-cell incubation during detection of *E. coli* O157:H7 provides ample time for cell lysis to occur. Cy5 CP1 has at least 10-fold more sensitivity than Cy5 SMAP but showed little activity in 30 minutes, causing only a $^1/_2$ to 1 log reduction. It is possible that the lysed cell fragments containing bound Cy5 SMAP were washed away during the assay. Bactericidal activity of Cy5 PGQ was not tested because its activity was greater than the 5 µg/ml used in the solution or immuno-capture assays. Potential activity from using concentrations greater than 5 µg/ml Cy5 CP1 need to be determined. Identification of peptides with high binding affinity and reduced activity would also be worthwhile candidates to investigate. Soares *et al* (Soares *et al.*, in press) demonstrated that the use of peptide fragments altered binding behavior compared to the parent peptide. This approach may be useful in finding candidates with desired binding and activity characteristics.

Decreasing overall assay time would be desirable and may be possible by reduction of the incubation period of labeled AMP with captured cells. Rapid binding of Cy5 CP1 and Cy5 SMAP to *E. coli* O157:H7 in as little as 5 minutes has been observed. Signal intensity is not significantly different than the signal resulting from the standard 30 minute binding (data not shown). Bactericidal activity in as little as 2 minutes has been reported for natural cecropin P1 against K12 strain *E. coli* D21 (Boman *et al.*, 1993) and SMAP-29 against *Pseudomonas aeruginosa* (Travis *et al.*, 2000). Since binding of these peptides is a precursor to cell lysis, rapid activity implies that binding is also occurring very quickly. A thorough investigation of how incubation time correlates with detection sensitivity has not yet been conducted.

Other Applications

Immuno-detection is a commonly used method for pathogen detection from a variety of sources. As a detection molecule, the fluorescently labeled peptide Cy5 CP1 has been shown to enhance sensitivity relative to a labeled antibody in buffer and apple juice, but not milk. While the impact of the samples matrix (e.g., food, environmental samples) on immuno-detection has been investigated with antibodies, it is unknown what effect it will have using peptides for detection. For food, complex samples such as ground beef will likely present additional challenges, although the effect may be minimized because the majority of interfering substances would be washed from the beads when peptide is added. AMPs may also have application in other biosensor detection formats where antibodies are currently used for detection. Cy5 labeled antibodies have been used in a fiber optic immuno-sensor to detect *E. coli* O157:H7 (DeMarco and Lim, 2001; Demarco and Lim, 2002), *Listeria monocytogenes* (Geng *et al.*, 2004), and *S. typhimurium* (Zhou *et al.*, 1997). In addition to fluorescence, *E. coli* O157:H7 and *S. typhimurium* detection with electrochemiluminescence has been demonstrated (Yu and Bruno, 1996). Colorimetric detection of immuno-captured *B. globigii* has been reported (Wiemer *et al.*, 2001). While the data presented here demonstrates improved detection, only a single organism has been examined. Although binding of fluorescent AMPs to other organisms has been demonstrated, detection limits need to be determined. Only then will the utility of labeled AMP's as an alternative molecule for enhanced detection be known.

4. CONCLUSIONS

Enhanced detection of *E. coli* O157:H7 has been demonstrated using a fluorescently labeled antimicrobial peptide in place of an antibody. Additional evaluation of labeled AMPs is needed in food samples. Assay optimization including improvement of signal to noise and reduction of assay time may further increase sensitivity. The combination the antibody specificity and increased signal provided by AMP detection molecules offers the opportunity to improve pathogen detection. Due to their ability to bind to other microorganisms, peptides have potential utility as to be used as enhanced detection molecules.

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14. COMMENTS:

PAO comments: under Abstract, end of 3rd sentence "Due of their small..." I think the word "of" should be changed to the word "to." Also, para. 3.4 Optimization - not sure you can fix, may be the template but their is a space between Opti mization and the same think in para. 3.5 Other Applications - the letter "r" of Other has an extra space.

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